

Figure 1.
Cloning vectors for the expression of UDP and PNP enzymes

RBS	EcoRI	KpnI	Sali	SphI	HindIII
AGCAAAACAGCT	ATG ACC ATG ATT ACG AAT TCG AGC TCG GTA CCC GGG GAT CCT CTA GAG TCG ACC TGC AGG CAT GCA AGC TTG				
thr met ile thr asn ser ser ser val pro gly asp pro leu glu ser thr cys arg his ala ser leu					

RBS *EcoRI* *SalI*

AGGAAACAGCT ATG ACC ATG ATT ACG AAT TCT TCC ATG GCT ACC CCA.....TGG GCG TAA AGAGTAAGTCACCTGC....
thr met ile thr asn ser ser met ala thr pro.....trp ala stop

RBS *KpnI* *SalI*
 AGGAARACAGCT ATG ACC ATG ATT ACG AAT TCG AGC TCG GTA CCA TCC ATG CTG TAA TTCTCTTGTGCAATG....
 thr met ile thr asn ser ser val pro ser met ser.....leu leu stop

SalI/NheI RBS EcoRI SalI SphI
GTCGACTAGCAGGAGGAATTCATG GCT ACC CCA..... TGG GCG TAA AGAGTAAGTCGACCTGCAGGCATGCAA
 met ala thr pro..... trp ala stop

Figure 2. 5' and 3' sequences of *udp* e *deoD* genes cloned in plasmid pUC18. Restriction sites of different constructs are underlined; the ribosome binding site (RBS) is reported in bold. The bases of nucleotide sequence of *udp* and *deoD* genes and the amino acid residues of PNP and Udp proteins are reported in italics.

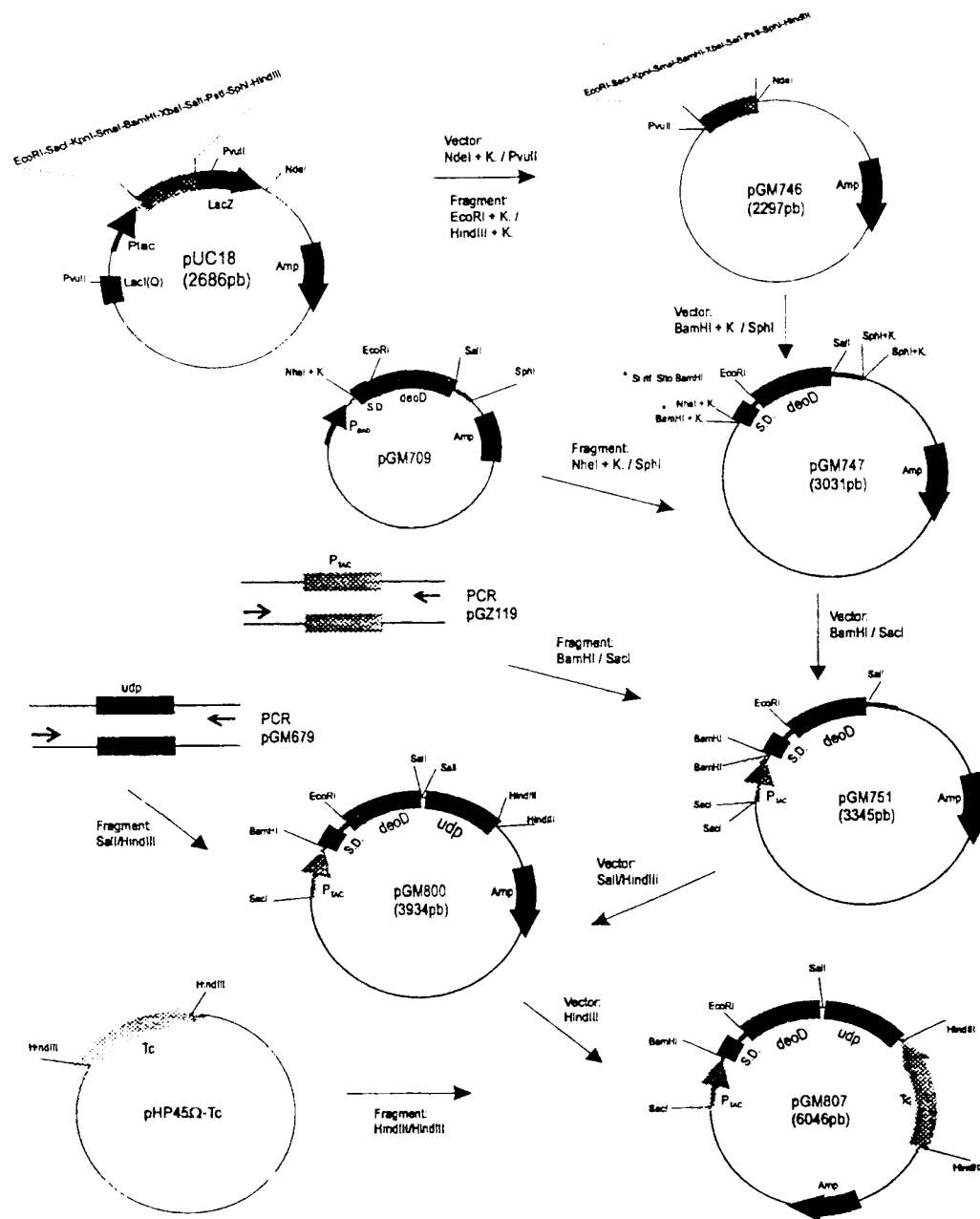


Figure 3.
Costruction of cloning vectors for the expression of UdP and PNP enzymes

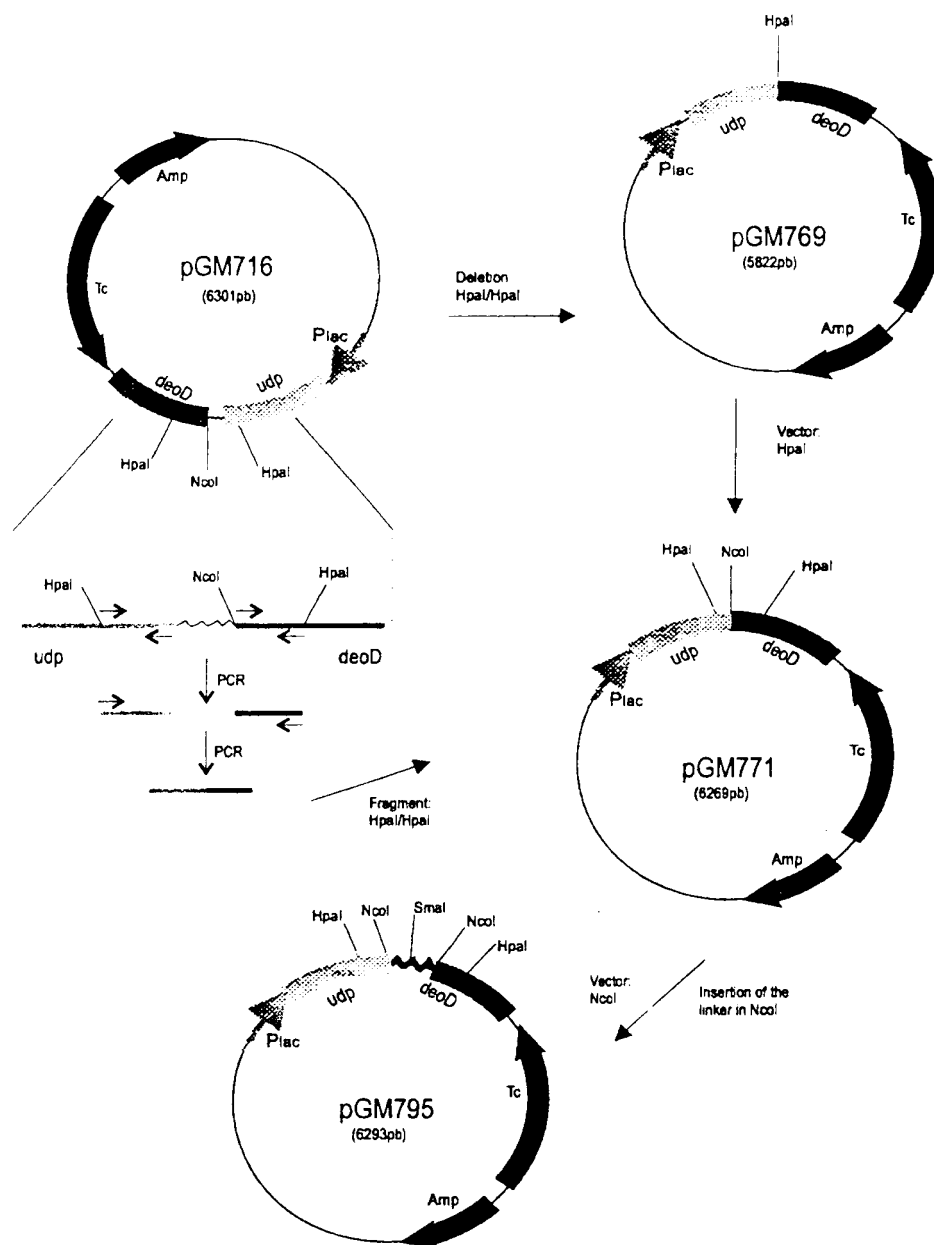


Figure 4.
Construction of cloning vectors for the expression of UdP-(L)-PNP enzymes.

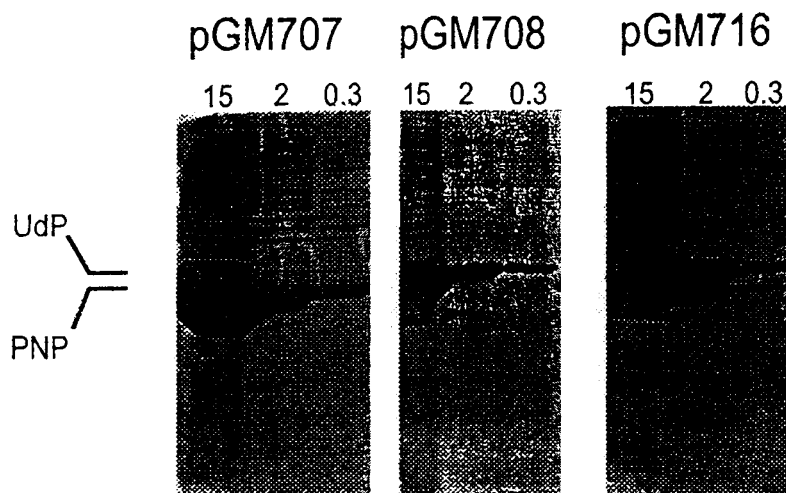


Figure 5.

Expression of PNP and UdP in recombinant *E. Coli* strains. Gel electrophoresis (SDS-PAGE) of total protein extracts from strains MG1655/pGM707, MG1655/pGM708 and MG1655/pGM716 grown over night in LD medium supplemented with 12.5 mg/liter of tetracycline. Lanes 15, 2 and 0.3 correspond to protein extracted from 15, 2 and 0.3 ml of bacterial culture.